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Research Note

Development of a *Sarcocystis*-like Apicomplexan Protozoan in the Brain of a Raccoon (*Procyon lotor*)

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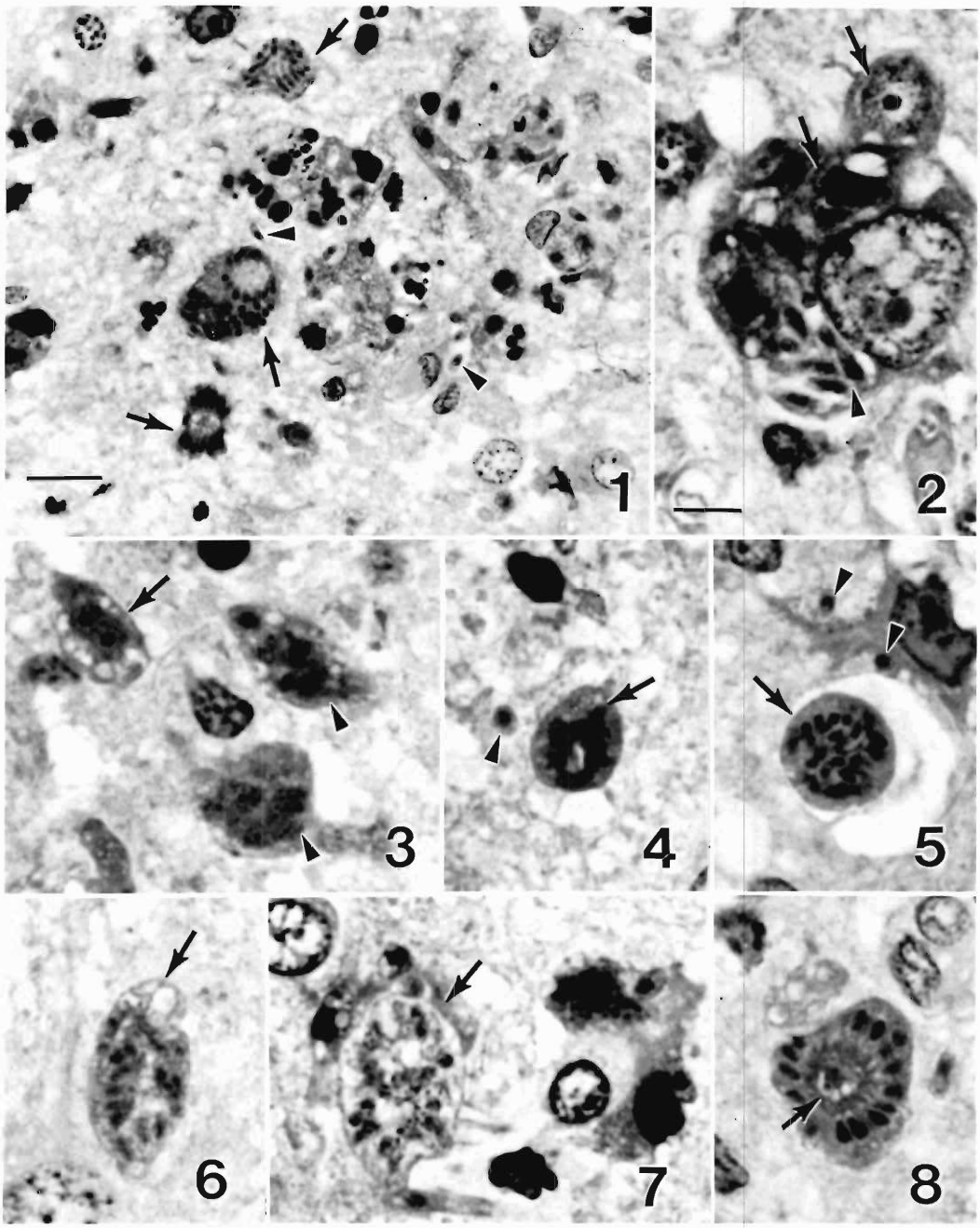
ABSTRACT: Schizonts of a *Sarcocystis*-like protozoan were found in the brain of a raccoon (*Procyon lotor*). The parasites, located directly in the cytoplasm of macrophages, neurons, and multinucleated giant cells, were not surrounded by a parasitophorous vacuole. The parasite divided by endopolygeny, leaving a residual body. Schizonts were 5–35 × 5–20 μm and contained up to 35 merozoites. The merozoites had no rhoptries. The parasite was antigenically and structurally similar to *Sarcocystis neurona,* the organism of equine protozoal myeloencephalitis.

KEY WORDS: Protozoa, Apicomplexa, coccidia, *Sarcocystis,* encephalitis, schizonts, merozoites.

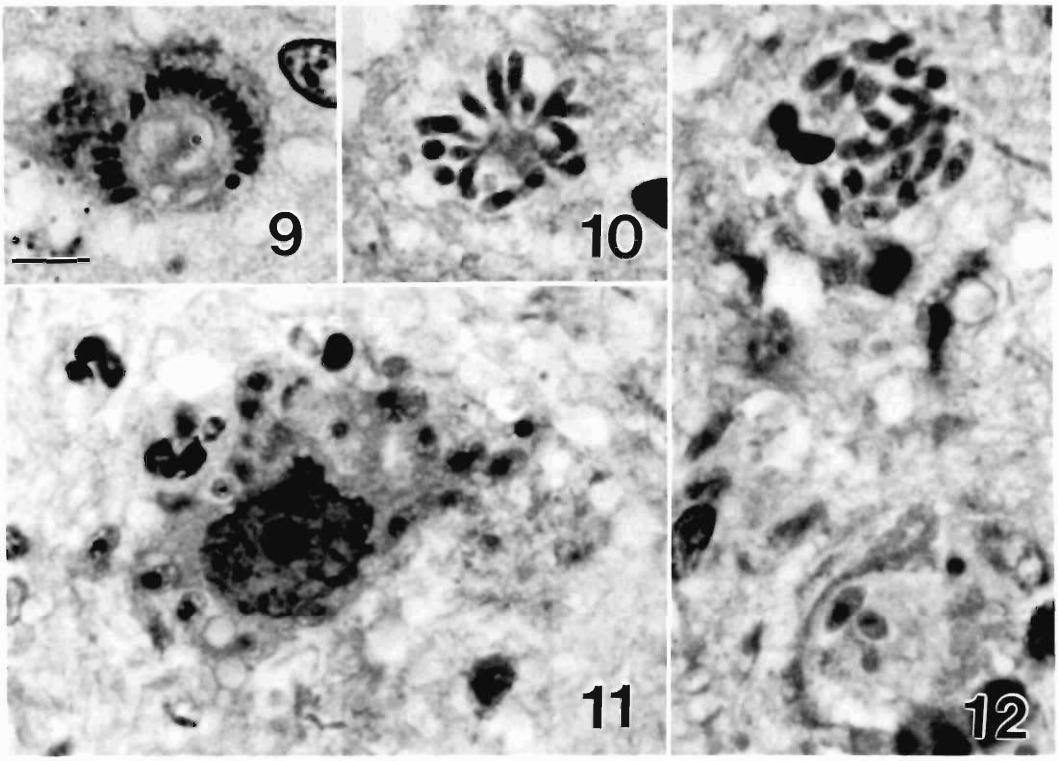
Toxoplasma gondii and *Neospora caninum* are the only known apicomplexan coccidians to cause fatal encephalomyelitis in carnivores (Dubey and Beattie, 1988). Recently Dubey et al. (1990) reported encephalitis in a raccoon associated with a *Sarcocystis*-like protozoan distinct from *T. gondii* and *N. caninum.* In this paper we report the development of the protozoan from the raccoon, *Procyon lotor* (L.), from Ohio.

Specimens of cerebrum were fixed in 10% buffered neutral formalin. Paraffin-embedded sections were cut at 3–6 μm, stained with hematoxylin and eosin (H&E), and examined microscopically. Selected specimens were embedded in glycol methacrylate and 2–3-μm sections were stained with H&E or periodic acid-Schiff hematoxylin (PASH). Formalin-fixed tissue was also processed for transmission electron microscopy. All measurements are given in micrometers.

Only asexual stages were seen (Figs. 1–12). Schizonts were located in neurons and macrophages. Individual merozoites were seen in neutrophils and mononuclear cells in lesions and in mononuclear cells in meningeal blood vessels. Most organisms seen were in macrophages. The



Figures 1–8. Stages of a *Sarcocystis* sp. in plastic-embedded sections of cerebrum of a naturally infected raccoon. 1. Individual merozoites (arrowheads) and developing schizonts (arrows) in a focus of necrosis. 2. Infected neuron with several schizonts. Arrows point to early schizonts with prominent nucleoli. Arrowhead points to mature schizont with merozoites. 3. Schizonts with undifferentiated nucleus or nuclei. Arrow points to a bilobed nucleus and arrowheads point to dividing nucleus. 4. Lobes of irregularly shaped nucleus. Arrowhead points to an extracellular merozoite. 5. Multinucleated schizont. 6. Schizont with a thin limiting membrane (arrow) and forming merozoites. 7. Schizont (arrow) with irregularly arranged developing merozoites. 8. Merozoites budding around a residual body (arrow). All figures H&E. Figure 1, scale bar = 13.3 μm, ×750; Figures 2–8, scale bar = 6.6 μm, ×1,500.



Figures 9–12. Schizonts and merozoites of a *Sarcocystis* sp. in plastic-embedded sections of cerebrum from a naturally infected raccoon. 9. Rosette of 19 merozoites. 10. Ruptured schizont with merozoites still attached to the residual body. 11. Numerous individual merozoites dispersed in the cytoplasm of a neuron. 12. Schizont with haphazardly arranged merozoites (above) and 2 merozoites in a cell (below). Merozoites in Figures 10–12 appear morphologically dissimilar, probably due to plane of sectioning and the stage of fixation. Figure 9, PASH; Figures 10–12, H&E. Scale bar = 6.6 μm. All figures ×1,500.

structure of the parasites was difficult to discern in thick (5–6 μm) H&E stained sections and because of multiple stages being present in a single cell (Fig. 2). More details were visible in 2–3 μm plastic embedded sections (Figs. 2–12).

Schizonts divided by endopolygeny, a divisional process similar to that in *Sarcocystis* schizonts (Dubey et al., 1989). The early schizont (7 long × 5 wide) contained a large nucleus with a prominent nucleolus. The nucleus became multilobed as the schizonts matured (Figs. 3, 4). Up to 35 nuclei or nuclear lobes were seen (Figs. 5, 6) and merozoites were formed internally as well as peripherally (Figs. 6–8). Sometimes merozoites were arranged in a rosette around an eosinophilic residual body (Figs. 8–10). However, not all merozoites were arranged peripherally (Figs. 11, 12). Individual merozoites were pear-shaped with a central nucleus (Fig. 12).

Ultrastructurally, all parasite stages were located in the host cell cytoplasm without a parasitophorous vacuole (Fig. 13–16). Host cells appeared to be fibroblasts and macrophages and some harbored multiple parasites in various

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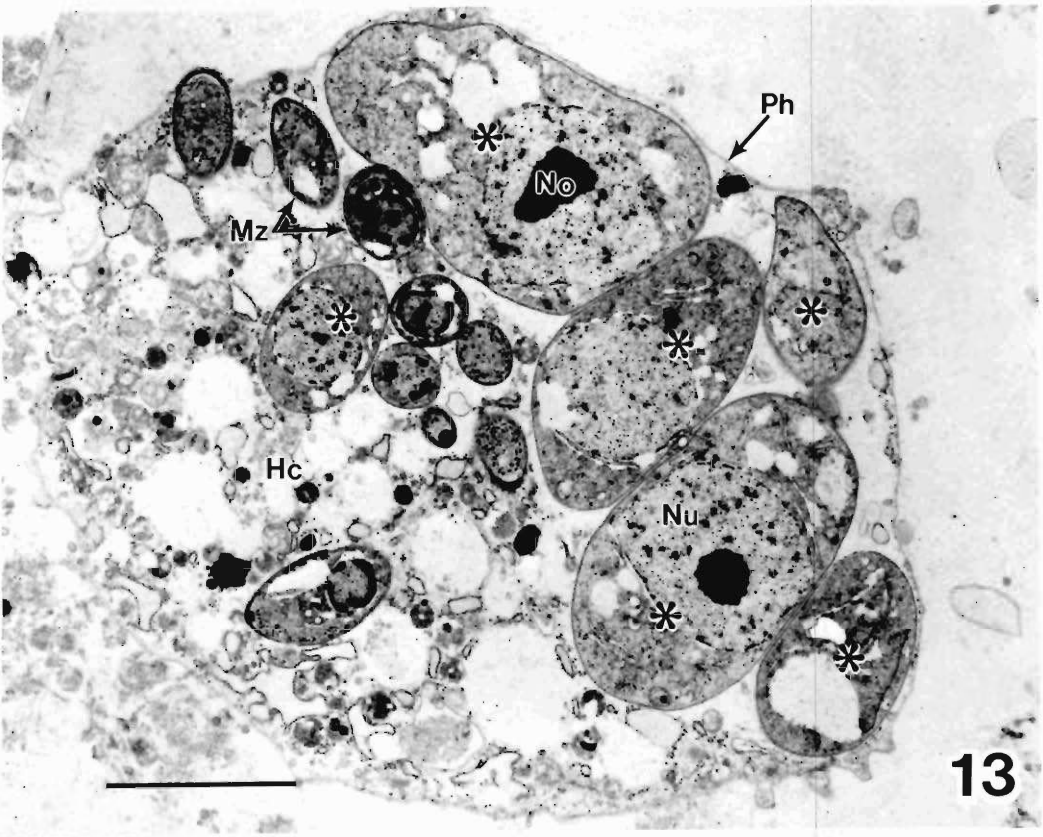
Figures 13, 14. Transmission electron micrographs of *Sarcocystis* sp. in cells of a raccoon. 13. Degenerate host cell infected with merzoites (Mz) and early schizonts (*). Hc, host cell cytoplasm; Ph, plasmalemma of host cell. Bar = 5 μm. ×5,000. 14. Intermediate schizont in early stage of merozoite formation. Ac, developing apical complex of merozoite; Er, parasite endoplasmic reticulum; Hc, host cell cytoplasm; Nu, nucleus of schizont; Ph, plasmalemma of host cell; PI, plasmalemma of schizont. Bar = 5 μm. ×7,500.



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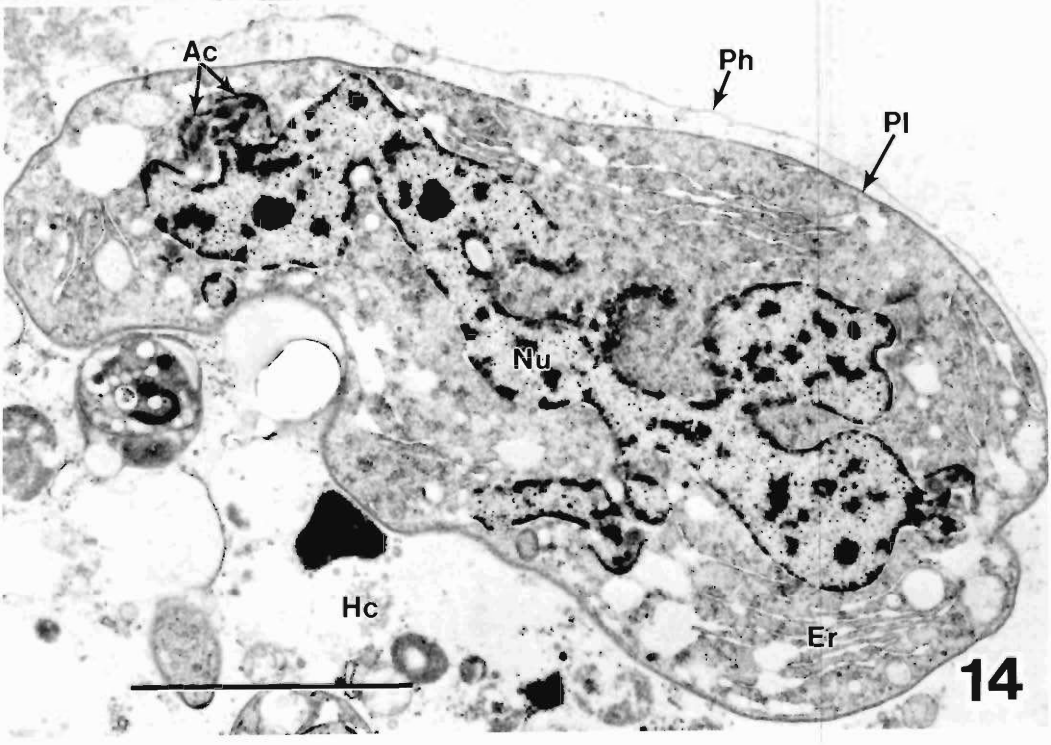

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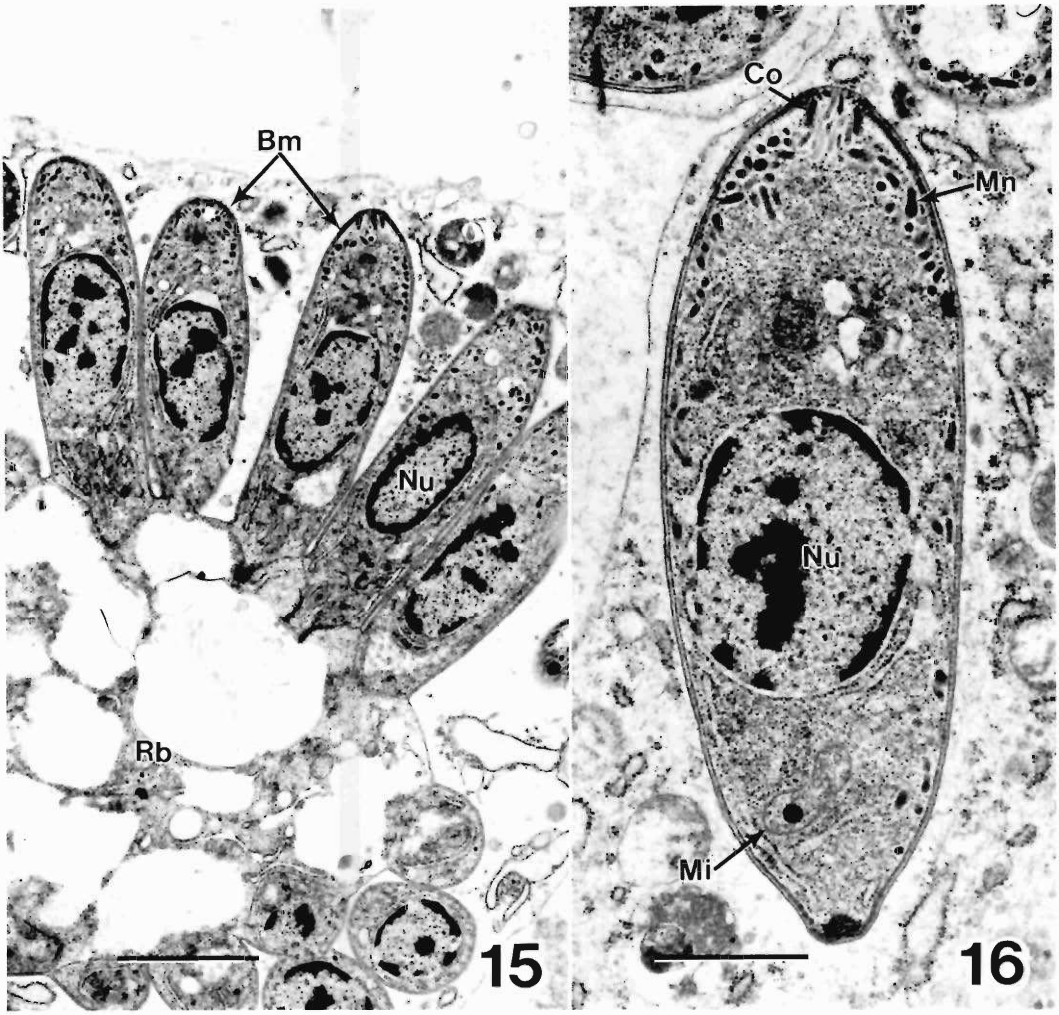

Figures 15, 16. Transmission electron micrographs of *Sarcocystis* sp. in cells of a raccoon. 15. Nearly mature schizont with merozoites (Bm) budding from a large centrally located residual body (Rb). Nu, merozoite nucleus. Bar = 2 μm. ×9,000. 16. Merozoite showing conoid (Co), mitochondrion (Mi), micronemes (Mn) and nucleus (Nu). Bar = 1 μm. ×20,000.

stages of multiplication (Fig. 13). The parasite multiplied exclusively by endopolygeny in which numerous merozoites began development internally and later budded simultaneously at the surface of the schizont. Young ovoid schizonts (14 long × 9 wide) contained a large nucleus with a single nucleolus plus other organelles characteristic of *Sarcocystis* spp. (Fig. 13). In more advanced schizonts, the nucleus became irregularly shaped with several nucleoli. Intermediate schizonts contained anlagen of developing merozoites and a highly lobulated nucleus (Fig. 14). A spindle apparatus consisting of several microtubules appeared in association with each lobe of the nucleus. Merozoite anlagen formed immediately above each spindle apparatus (Fig. 14). Each merozoite anlage elongated by *posterior extension* of its inner membrane complex and subpellicular microtubules and eventually incorporated within it a part of the nucleus and cytoplasm. The pellicular membrane folded in around the developing merozoites until they appeared to bud at the surface of the schizont (Fig. 15). Schizonts contained approximately 50–60 merozoites. The merozoites measured from TEM photographs were 5.8 × 1.8 (5.4–6.0 long × 1.6–2.0 wide; *N*

= 12) and contained all of the organelles and inclusion bodies characteristic of a species of *Sarcocystis* (Dubey et al., 1989). Rhoptries were absent (Fig. 16).

The parasite in the raccoon was not *T. gondii* or *N. caninum* because it divided by endopolygeny, was not located in a parasitophorous vacuole, and did not react with antisera against *T. gondii* and *N. caninum* in an immunohistochemical test (Dubey et al., 1990). Structurally, the raccoon parasite appears identical with the newly named organism, *Sarcocystis neurona* Dubey, Davis, Speer, Bowman, de Lahunta, Granstrom, Topper, Hamir, Cummings, and Suter, 1991, that causes fatal neurological disease in horses (Fayer and Dubey, 1987; Dubey et al., 1989, 1991).

The life cycle of *S. neurona* is not known. Only schizonts in the central nervous system of horses have been found. *Sarcocystis neurona* was recently grown in bovine monocytes in culture (Dubey et al., 1991). Further studies in the raccoon and with cultured organisms might help elucidate the life cycle of these parasites that cause encephalomyelitis in animals.

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Research Note

Helminth Parasites from Some Tichigan Lake Fishes in Southeast Wisconsin

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ABSTRACT: A total of 2,525 fishes of 30 species from Tichigan Lake and associated waters (Racine County) was examined for parasites between 1977 and 1979. *Corallobothrium fimbriatum, C. giganteum, Ophiotaenia fragilis, Proteocephalus* sp. (Cestoda), and *Polylekithum ictaluri* (Trematoda) are reported from *Ictalurus punctatus,* and *Triaenophorus nodulosus* plerocecoids from *Catostomus commersoni.* All records are new to southeastern Wisconsin. Channel catfish appeared to be the major host of *C. giganteum. Corallobothrium* recruitment occurred during all seasons, but was maximal during autumn. Development, maturation, prevalence, and intensity of infection increased during spring and summer. Infection with *Corallobothrium* was not associated with host sex or size; no posterior migration was observed. The white sucker appears to be the more common host of *T. nodulosus* in North America. The seasonality of *Acanthocephalus dirus* (Acanthocephala) in 9 common fish hosts was similar to that of *Corallobothrium.* Infections in 1977–1979 were, however, very light and fecundity unusually low. The spring population was compared with those from other years, e.g., 1984, and from a riverine habitat, the Pike River.

KEY WORDS: Wisconsin, catfish, *Corallobothrium, Ophiotaenia, Proteocephalus, Polylekithum, Triaenophorus, Acanthocephalus,* seasonality, host size and sex, site selection.

The seasonal ecology of cestode parasites of catfish, and of *Acanthocephalus dirus* from 9 fish